

Protein extraction and ELISA

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 An abbreviated version of this protocol was published in Science Immunology in May 2022

Intratumoral immunotherapy relies on B and T cell collaboration

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Detailed protocol

Mouse plasma production-

Collect samples in EDTA tubes.

Collect blood from mice cheek (submandibular), centrifuge samples at 500 x g at room temperature for 10 minutes (cells in pellet). Collect the supernatant and transfer into a clean tube. Centrifuge supernatant at 13,000 x g at room temperature for 10 minutes. Aliquot and use immediately or store at -80°C.

Tumor tissue supernatants production-

Anesthetize mice using CO₂ or isoflurane, followed by cervical dislocation. For tumors inoculated in the abdomen, gently remove the abdominal skin without tearing the peritoneal cavity.

Remove the local draining lymph node using forceps. Gently separate the tumor from the skin using forceps and surgical scissors. Transfer the tumor to a 6mm dish containing 1mL of tissue protein extraction reagent and 1% proteinase and phosphatase inhibitors. Mince the tumors using surgical scissors and transfer the tumor lysates into a 15 ml tube using. Incubate at 4°C for 30 min with slow rotation and then centrifuge for 15 min at 13,000 x g to remove debris.

Aliquot and use immediately or store at -80°C.

Levels of IL-12 and IFN- γ in tumor tissue supernatants or in plasma were measured by ELISA kits (R&D Systems), following the manufacturer's instructions.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Sagiv Barfi, I. (2022). Protein extraction and ELISA. Bio-protocol Preprint. bio-protocol.org/prep1794.
2. Sagiv-Barfi, I., Czerwinski, D. K., Shree, T., Lohmeyer, J. J. K. and Levy, R. (2022). Intratumoral immunotherapy relies on B and T cell collaboration. Science Immunology 7(71). DOI: [10.1126/sciimmunol.abn5859](https://doi.org/10.1126/sciimmunol.abn5859)

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